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(6) PHYSICAL AND BIOCHEMICAL STUDIES
ON
THE MICROSOMES AND ITS NUCLEIC ACID.

RESPONSIBLE INVESTIGATOR

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PHYSICAL AND BIOCHEMICAL STUDIES
ON
THE MICROSOMES AND ITS NUCLEIC ACID

The Semi-Annual Report

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I. THE PURPOSE OF THIS INVESTIGATION IN THE THIRD YEAR

1. Studies have been made on some physicochemical properties of liver ribosomes during the past two years. In the last year, we isolated brain ribosomes from microsome as well as from nuclear fraction. However, the comparison of brain ribosomes with liver ones, have not been completed due to technical difficulties of the purification procedure. We intended to purify ribosomes from brain microsome.

2. In the course of study on nuclear ribosomes from rabbit brain, we found large impurity in "nuclear" fraction by means of electronmicroscopy. The isolation method of brain nucleas from homogenate will be also continued to study.

II. RESULTS OBTAINED TO DATE

1. The Purification of Brain Ribosomes.

Liver ribosomes were isolated by the procedure same as that described in the previous report. Crude brain ribosomes were prepared by the same procedure as that of crude liver ones but with slight modification. Preliminary experiments revealed that high concentration of magnesium ions as was used to purify liver ribosomes (e.g. 50 mM $MgCl_2$ solution) could distort brain ribosomes. This is one of the difficulties of purification. After trials, it was found 0.01 M $MgCl_2$ solution adequate. The crude preparation of brain ribosomes was suspended in 0.01 M $MgCl_2$ solution, after centrifugation the sediment was dissolved in 0.02 M Tris buffer and dialysed overnight. The dialyzate was spun at 60,000 g for 15 minutes, the supernatant obtained was centrifuged at 105,000 g for 3 hours. The gelatiniform pellet, purified ribosomes, was thus obtained.

The purified brain ribosomes were characterized by four peaks of sedimentation constants of 120 s, 80 s, 60 s and 40 s in centrifugal analysis, a finding quite similar to that on liver ones (Takanami, 1960). Molecular weight of the 120 s, 80 s, 60 s and 40 s particles are determined as 6.9×10^6, 3.7×10^6, 2.3×10^6 and 1.3×10^6 respectively, by means of sedimentation constant-molecular weight relation proposed by Inouye et al. (1963) Four types of particles are readily visible in electronmicrograph. Ultraviolet absorption spectrum of the preparation showed characteristics of ribonucleoproteins. The nearly constant ratio of RNA to protein of 1.05 (average of eight experiments) was determined, this ratio of liver ribosomes was 0.67 with the same technique.

2. The isolation of Brain Nuclei.

Applying the method of density gradient centrifugation, nuclear fraction obtained was of about 90 % pure in a phase microscope. However, it was found to be pretty impure by electron microscope examination. As was reported in the Final Report No. 2, isolation of nuclear ribosomes was very difficult. One of causes of the difficulties may be the impurity of nuclear fraction. The further study will be made in the next period.

III. RESEARCH PLAN AT THE NEXT PEPTOD

1. The observation on the effect of magnesium ion concentration on calf brain ribosomes.

   The purification method of brain ribosomes was established in this period, some observation stated above will be repeated on the stability of ribosomes.

2. The neurochemical and neurophysiological characterization of brain microsome.

   Electron microscope observation on brain microsome was already done in our laboratory (Shinagawa et al. 1963), components of brain microsome except ribosomes (esp. membrane of endoplasmic reticulum) will be studied.

3. The purification of brain nuclei to establish the foundation of isolation of brain nuclear ribosomes.

IV. LIST OF REFERENCES

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